CONTROL OF FLASHING IN FIREFLIES. III. PERIPHERAL EXCITATION

JOHN BUCK, JAMES F. CASE[1] AND FRANK E. HANSON, JR.

Laboratory of Physical Biology, National Institutes of Health; Department of Biology, University of California, Santa Barbara; Department of Zoology, University of Pennsylvania; and Marine Biological Laboratory, Woods Hole, Mass.

In preceding papers (Buck and Case, 1961; Case and Buck, 1963; hereinafter designated "FF-I" and "FF-II") we described responses of five species of lampyrid firefly to electrical stimulation and explored the role of the central nervous system in excitation and modulation of luminescence. Some of these observations also bear on properties of peripheral parts of the excitation pathway. For example, we found that both normal spontaneous flashing and that triggered by electrical stimulation of the brain are excited by characteristic volleys of action potentials detectable in the cord and also throughout lantern tissue, presumably in peripheral nerve (FF-II). Likewise, the finding that only about a third of the variation in flash intensity is demonstrably dependent on variation in associated neural signals (FF-II) suggests the possibility of modulation at neuroeffector junction or photocyte level.

Among other indications that the eventual production of a flash involves more than an electrical signal and a conduction pathway direct to the photocyte are the facts that photic volleys can be recorded at a stage of hypoxia that prevents flashing (Carlson, 1961) and that flash intensity can be influenced by "priming" stimulation and by volley frequency, both of which involve effects persisting far longer than one would expect of purely neural facilitation (FF-I, II).

One of our most provocative findings is that response latency is long. In *Photuris* the delay between time of stimulation in the head and the resulting flash is usually 90–120 milliseconds (msec.) at 20–25° C. Even with the electrode pair directly in photogenic tissue, latency is between 65 and 85 msec. and up to 50 msec. can elapse between the last spike of the normal photic volley and the start of luminescence. It thus takes more than twice as long for excitation to pass from cord to photocyte, a distance that cannot exceed about 2 mm., as to traverse the entire 8–10-mm. length of the cord. The cord-to-photocyte latency undoubtedly includes not only conduction delay in peripheral nerve fibers but delay in some sort of neuroeffector junction.

The only available measurement bearing directly on peripheral conduction velocity is the 5–15-msec. delay between the time a spontaneous photic volley is detected in the posterior cord, dorsal to the lantern, and the time of its arrival on the ventral surface of the photogenic tissue (FF-II). The point in the peripheral innervation from which such potentials are recorded is not known exactly, but it probably lies near to the distal nerve endings, hence indicating that only

[1] Aided by N.I.H. Grant B-1890.

a few milliseconds are occupied in actual conduction with the lantern. This deduction is also supported by the fact that the volley-flash delay in spontaneous flashing is so nearly the same as the stimulus-flash latency in flashing induced by direct stimulation of the lantern (FF-I, II). Further, pair-shock experiments demonstrate that detectable augmentation of response can be produced by a test shock occurring at any time from a few milliseconds after the conditioning shock to a few milliseconds before the flash begins (FF-I). However, in view of the lack of direct information about size and pathway of the finer fibers and the nature of their endings, it is not excluded that conductional delay is a major fraction of overall peripheral latency. The present paper is accordingly devoted to trying to define more precisely the sequence and path of peripheral excitation.

Materials and Methods

The lantern of the adult firefly consists of a flat luminous organ just inside the ventrical cuticle of both of abdominal segments 6 and 7. They are innervated from ganglia 4–6 of the ventral cord. The larval organs are a pair of small discs located ventrolaterally in the 8th abdominal segment. Methods for stimulating light production electrically and for recording the light emission are given in our two preceding papers. Unless otherwise noted the responses described in this paper were evoked via an electrode pair of 0.005-inch bare silver wires placed directly in lantern tissue, 1–2 mm. apart. The stimulus source was a Grass S-4 stimulator connected through a stimulus isolation unit. The nominal stimulus voltages used in the various experiments have comparative value only, since tissue resistance was not measured and we were in any case dealing with a multicellular response system in which overall potential difference gives little indication of actual current flux per cell. Further details concerning species of firefly studied and methodology are given in FF-I and FF-II.

Results

1. *Peripheral excitation pattern*

The normal spontaneous firefly flash is so brief and brilliant that it is extremely difficult to make out local details, but excitation usually appears to be simultaneous and uniform over the entire area of the lantern. This is true also for the vast majority of driven flashes. Occasionally, however, spontaneous or induced flashes may appear only in certain parts of the lantern or may involve slight asynchrony between different areas of photogenic tissue (see also FF-II). If only part of the lantern is active it is apt to be the central portion of the sixth sternital organ, the area of luminescence often increasing with successive flashes. However, lateral regions may upon occasion flash without the central portion. Many of these local responses can be interpreted in terms of the gross distribution of the peripheral nerves (Hanson, 1962).

In photurids that were emitting mixtures of single, double, and triple spontaneous flashes a 0.005-in. thick brass sheet, pierced by three 200-μ holes about 800 μ apart, was applied closely to the lantern in such a way that one hole lay over the center of the anterior edge of the segment 6 organ, one lay over the posterior edge of the same segment, and the third lay over the posterior edge of the seventh

segmental organ. By covering the holes two at a time and recording the light passing through the third it was shown that each of the three widely separated areas of the lantern, each amounting to less than 1/50 of the total area, was able to produce single, double, and triple flashes. Though quite variable in contour the multiple flashes were comparable in peak timing to those recorded from the whole unmasked lantern (FF-II, Fig. 2). This suggests that usually the sequence of spontaneous excitation of the lantern is similar in all areas. The same conclusion was indicated by the fact that similar action potentials can be recorded from different sites in the lantern (*e.g.*, FF-II, Fig. 32).

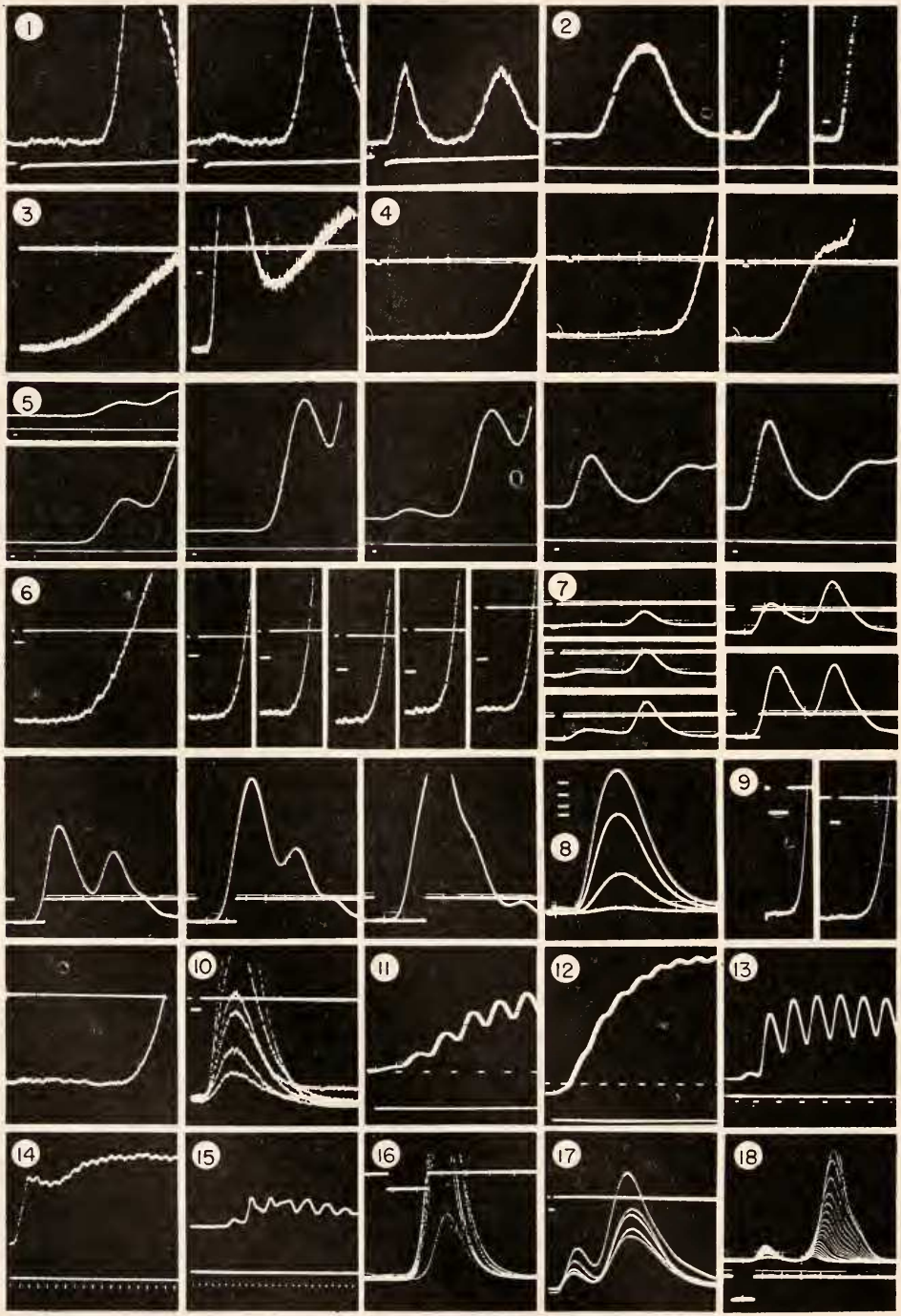
2. *Short latency response of adult*

Adult fireflies at room temperature ordinarily respond to shocks of only a few volts intensity and a few msec. duration, particularly if stimulated directly in the lantern. When stimulus strength reaches quite high values a new short latency response often appears instead of or in addition to the usual flash. This new response we call a "quick flash" to distinguish it from the usual longer latency "slow flash" described in the first two papers of this series.

Figures 1, 3, 7 and 9 illustrate quick flashes induced in various species by increasing stimulus duration, and Figures 2, 4, 5 and 6 the same for increasing voltage. Figure 5 shows the development of the quick flash in a species with multi-peak slow flash (*P. punctulatus*). The apparent merging of slow and quick flashes seen in some figures (*e.g.*, 2, 4, 6), as contrasted with records in which the responses remain distinct (Figs. 1, 3, 5, 7), can be brought about by excessive quick flash duration due either to high amplification of the light trace or strong stimulation.

As we shall show, the interrelations of slow and quick flash are quite complex. In a general sort of way, however, the slow flash is the characteristic response to weak or moderate stimulation, the quick flash is generally dominant with very strong stimulation, and both responses may occur together at intermediate stimulus intensities. Both types of flash may apparently involve the total lantern surface; however, because of the short interval between quick and slow flashes and because of the diffusion of light in all directions through the lantern tissue this is a point about which it is very difficult to be certain. For the same reasons we have as yet no certain answer as to whether both quick and slow flashes can be produced in sequence by the same cell. There were one or two instances in which quick and slow flashes were apparently confined to different segmental organs, but this is not necssarily indicative of a separate photocyte population for each type of response since stimulus current density may have been subliminal in one region and not in the other or one response may have been fatigued (see below).

A. *Threshold.* Stimulus duration and voltage thresholds for quick flash induction vary somewhat from individual to individual and species to species but often appear to be quite sharp in a given individual (Figs. 1–4). However, if levels are graded sufficiently closely it is usually possible to show that the quick flash builds progressively with increasing vigor of stimulation (Figs. 5–7) and that the effect is reversible (Fig. 9). Threshold not uncommonly falls with repeated stimulation, perhaps indicating facilitation. For example, whereas the quick flash might appear first at a nominal 20 msec./150 volts (in *Photuris*), only



FIGURES 1–18.

10 msec./60 V. might be sufficient after several successive trial series. Nevertheless it is sometimes possible to obtain reasonably conventional strength-duration data from individuals (*e.g.*, Fig. 19).

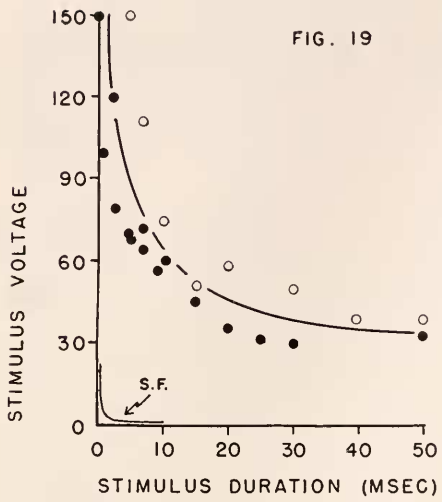
Quick flash thresholds for the small species, *Photinus marginellus* and *P. consanguineus*, are generally much lower than for *Photuris* and the large species of *Photinus*. This and other evidence discussed below indicate that threshold is importantly influenced by tissue mass and electrode placement. For this reason no reliance can be placed on our absolute threshold values. Since, however, the conditions of stimulation for slow and quick flash in the same species should be at least roughly comparable, the values of 10 msec. for chronaxie and 30 volts for rheobase for the quick flash of *Photuris* (Fig. 19) probably form a valid contrast with the corresponding slow flash values of 3.9 msec. and 2.1 volts (FF-I).

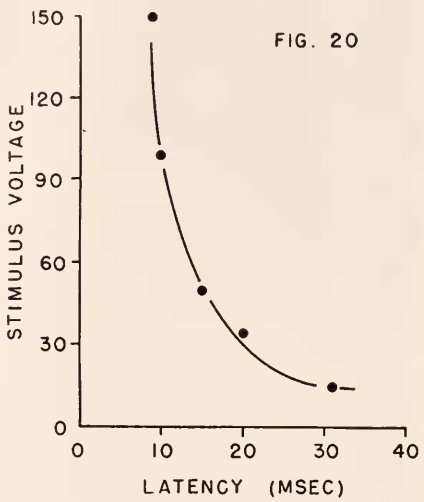
Stimulus strength has to be increased drastically in order to elicit slow flashes at low temperatures, and no slow flashes have been detected in *Photuris* below about 8° C. (FF-I). In contrast, excitation threshold of the quick flash seems nearly or quite independent of temperature, and responses have been elicited down to at least 3.5°, a temperature at which slow-flashing is probably impossible (*i.e.*, latency is infinite: Fig. 23; FF-I, Fig. 45).

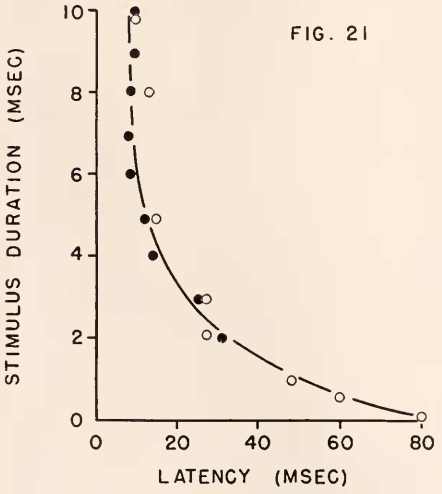
B. *Latency.* The most striking and characteristic difference between slow and quick flash is in latency. In *Photuris* (Figs. 1, 7), *Photinus pyralis* (Fig. 17), *P. punctulatus* (Fig. 5) and possibly in *P. consanguineus* (Fig. 24) there is

---

FIGURES 1–18. (In these and other oscillographs the time scale is from left to right and is given as S = entire width of picture in msec. Figures identified "As X" refer to the same individual as that of Figure X. Some figures slightly retouched.) (1) Woods Hole *Photuris*, male, isolated 6th segment organ. Three successive responses to shocks of 5 msec./150 V., 7/150 and 10/150. S = 150. (2) *Photinus marginellus*, male, isolated lantern. Three successive responses to shocks of 10 msec./7 V., 10/10 and 10/12.5. S = 875, 225, 225. (3) *Photinus consanguineus*, male, intact. Two successive responses to 5 msec./150 V. and 10/150. S = 440. (4) As 3. Three successive responses to 5 msec./20 V., 5/25 and 5/30. S = 175. (5) *Photinus punctulatus*, male, isolated 6th segment organ. Six successive responses to 1 msec./20 V., 1/40, 1/60, 1/80, 1/100 and 1/120. S = 175. (6) *Photinus marginellus*, male, decapitated. Six successive responses to 5 msec./40 V., 5/60, 5/80, 5/100, 5/120 and 5/150. S = 85, 30, 30, 30, 30, 30. (7) Iowa *Photuris*, male, isolated abdomen. Eight successive responses to duration series of 5, 7.5, 10, 15, 20, 30, 40 and 50 msec., voltage probably 150. S = 175. (8) *Photinus consanguineus*, male. Four superimposed successive responses to 10 msec./60 V., 10/90, 10/120 and 10/150. Stimulus artifacts above Figure number. S = 175. (9) Iowa *Photinus pyralis*, male. Three successive responses to 10 msec./150 V., 5/150 and 1/150. S = 50, 50, 92. (10) Iowa *Photinus pyralis*, male, decapitated. Six superimposed successive responses to 20 msec./150 V. S = 430. (11) Iowa *Photinus pyralis*, male. Response to train of 10 msec./150 V. shocks at 7/sec. S = 850. (12) As 11, but 10/sec. S = 850. (13) *Photinus punctulatus*, male, isolated 6th segment organ. Response to train of 2 msec./70 V. shocks at 30/sec. S = 250. (14) *Photinus punctulatus*, female, isolated organ. Response to train of 5 msec./50 V. shocks at 20/sec. S = 950. (15) *Photinus punctulatus*, male, isolated 6th segment organ. Response to train of 1 msec./15 V. shocks at 30/sec. S = 850. (16) Woods Hole *Photuris*, female, recording from 7th segmental organ in otherwise intact animal from which 6th segment organ had been removed. Superimposed responses to 20 successive shocks of 30/msec./150 V. at 2/sec. S = 145. (17) Iowa *Photinus pyralis*, male, decapitated. Five successive responses to shocks of 20 msec./150 V. S = 860. (18) Iowa *Photuris*, male, isolated abdomen. Twenty successive responses to 20 msec./150 V. at 2/sec., superimposed. In this series the quick flash augmented somewhat with repeated stimulation while the slow flash decreased progressively in intensity. S = 175.







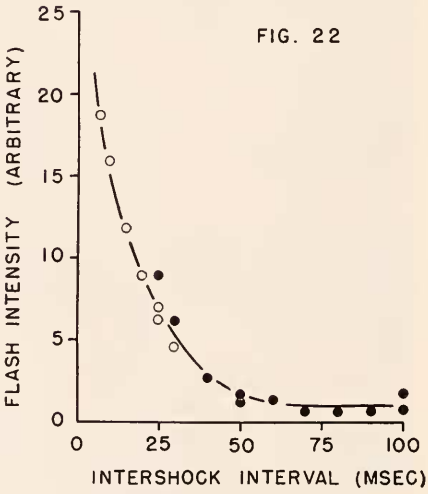


FIGURE 19. Strength-duration relations for quick flash threshold in two representative individuals. Solid circles, Woods Hole *Photuris,* male. Open circles, Maryland *Photinus pyralis,* male. "SF" indicates corresponding curve for slow flash of *Photuris* (from FF-I).

FIGURE 20. Relation between quick flash latency and voltage of stimulus. *Photinus consanguineus,* male.

FIGURE 21. Relation between quick flash latency and duration of stimulus. Solid circles, *Photinus consanguineus,* male. Open circles, Iowa *Photinus pyralis,* male.

FIGURE 22. Facilitation of quick flash by paired shocks. *Photinus punctulatus,* male. Solid circles, first run. Open circles, repeat run.

usually no question of the independence of the two phenomena, although the actual latency values may appear to vary somewhat with flash magnitude because of uncertainty in deciding the exact initiation point (*e.g.,* Fig. 4b). For this reason, also, latency may appear to be shorter with higher stimulus voltage (Fig. 8) or duration (Figs. 3, 7, 9). However, in the high intensity range of stimulation, when the lantern is well facilitated, latency often appears to be independent of flash amplitude (Figure 6). Thus with incremental changes in voltage (Fig. 20) and duration (Fig. 21), latency tends to approach a limiting minimum value. From such measurements it was possible to compile the quick flash latency estimates given in Table I.

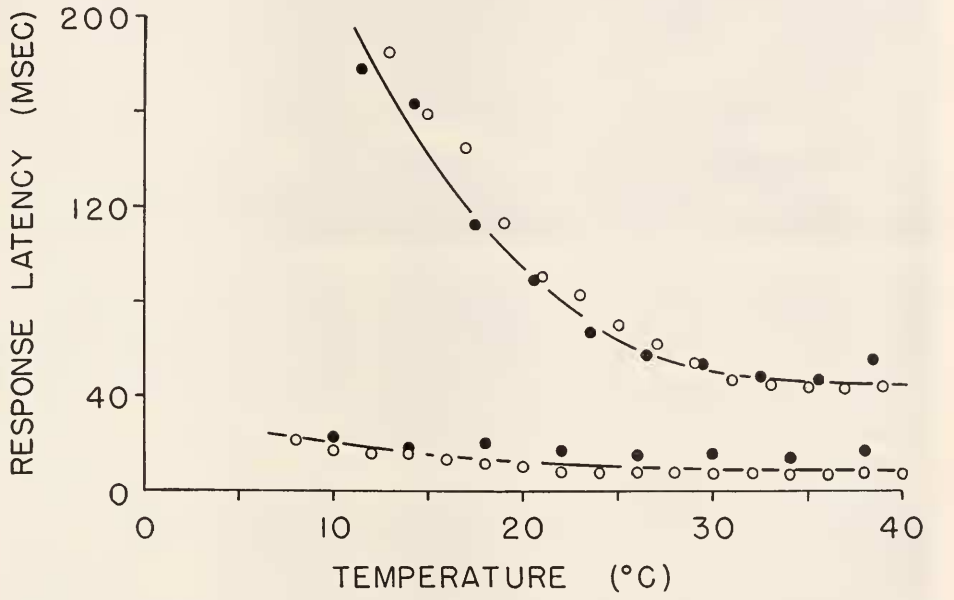
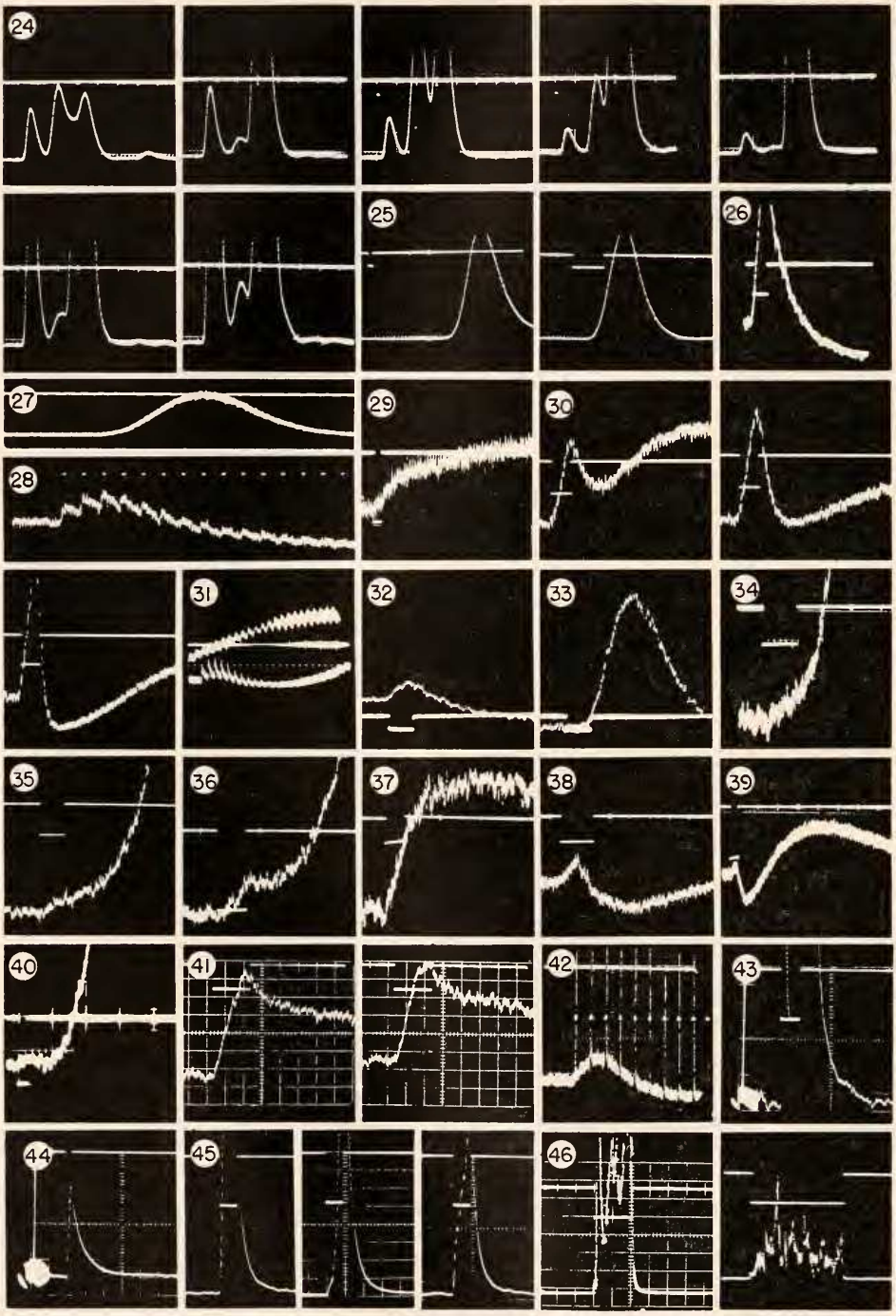


FIGURE 23. Latency in relation to temperature. Solid circles, *Photinus punctulatus.* Open circles, Woods Hole *Photuris.* Quick flash data: means for 113 measurements from 6 males of *P. punctulatus* and 37 measurements from 6 females of *Photuris.* Slow flash data (from FF-I): 73 measurements from 6 males of *P. punctulatus;* 145 measurements from 10 females of *Photuris.*

Quick flash latency scarcely varies with temperature (Fig. 23). In the 10–30° range the Q_{10} for the slow flash varies between 1.9 and 2.2 whereas that for the quick flash ranges from 1.3 to 1.6. It is this fact which furnishes perhaps the clearest evidence of a basic mechanistic difference between the excitation processes of the slow and quick responses.

C. *Effects of repetitive stimulation.* Like the slow flash, the quick flash may augment with repetitive stimulation (Fig. 10) and may simulate the fusion phenomena of striated muscle (Figs. 11 and 12). As with the response of the slow flash to train simulation, there may be considerable differences in tetanizing frequencies between species (Figs. 12, 13) and even, apparently, between the sexes



FIGURES 24–46.

TABLE I

Approximate peripheral response latencies (msec.; 25°)

Species	Ultra-short	Quick flash	Slow flash*	Ultra-long
Woods Hole *Photuris*	1	18	70	
Maryland *Photinus pyralis*		20	160	
Photinus punctulatus		15	75, 110**	
Photinus consanguineus		10	100, 175†, 250†	
Photinus marginellus		5–10***	40–200	
Maryland and Iowa *Photuris* larva	1–3			600

* Values in FF-I, Table I, are for 22° as stated in text, not 25° as erroneously given in table heading.

** Differs with sex—See FF-I.

*** Slow flash values of 26 and 27 msec. given in FF-I, Table I, now thought to have been incompletely facilitated quick flashes.

† Secondary peaks, not appearing alone.

(Fig. 13 vs. 14). Paired-shock experiments indicate the same general course of facilitation as for the slow flash, with 10–15 msec. estimated as the intershock interval for maximum effect in *P. consanguineus* and less than 7 msec. in *P. punctulatus* (Fig. 22).

Repetitive evocation of quick flashes often induces a continuous homogeneous background glow just as does train stimulation of slow flashes (FF-I: Figs. 33–35, 37, 51, 62–66) and in fact most of the phenomena dealt with in the present paper were recorded against a background of continuous luminescence rather than of complete darkness. With both quick and slow flashes the detectability of individual

FIGURES 24–46. (24) *Photinus consanguineus,* male, decapitated. First four frames are 1st, 3d, 5th and 7th consecutive responses to 5 msec./40 V. Last three frames are immediately following responses to 5 msec./50V., 5/60 and 5/70. S = 875. (25) As 16. Responses to 5 msec./15 V. and 30/150. S = 175. (26) Woods Hole *Photuris* larva, decapitated. Response to 30 msec. /150 V. S = 375. (27) As 26. Response to 10 msec./15 V. S = 2400. (28) Woods Hole *Photuris* larva, isolated organ. Response to train of 10 msec./100 V. shocks at 10/sec. S = 1750. (29) Iowa *Photuris* larva. Responses to (probably) 50 msec./150 V. S = 1080. (30) As 28. Three responses to 100 msec./150 V. S = 860. (31) Maryland *Photuris* larva, isolated organ. Response to train of 7 msec./150 V. shocks at 5/sec. Two sweeps (left end of upper trace continuous with right end of lower). S = 5500. (32) *Photinus consanguineus,* male, isolated 7th segmental organ. Response to 30 msec./150 V. S = 230. (33) As 32 except 6th segmental organ. (34) *Photinus consanguineus,* male, isolated abdomen. Response to 20 msec./150 V. S = 115. (35) *Photinus marginellus,* male intact. Response to 10 msec./150 V. S = 75. (36) *Photinus marginellus,* male isolated organ. Response to 10 msec./150 V. S = 75. (37) *Photinus consanguineus,* male, excised 6th segmental organ. Response to 10 msec./150 V. S = 88. (38) *Photinus marginellus,* male, decapitated. Response to 30 msec./150 V. S = 180. (39) *Photinus marginellus,* male. Response to 80 msec./150 V. S = 1750. (40) *Photinus punctulatus,* male, isolated 6th segmental organ. Response to 2.5 msec./100 V. S = 52. (41) Iowa *Photinus pyralis,* male, one half of one segmental organ freed of other viscera. Two successive responses to 10 msec./150 V. S = 47. (42) Iowa *Photinus pyralis,* male. Response to train of 10 msec./150 V. shocks at 12/sec. S = 925. (43) As 42. Response to 1 msec./150 V. S = 9.2. (44) As 43. Response to 0.1 msec./150 V. S = 4.5 (very fast sweep). (45) Moist filter paper. Three "responses" to 1 msec./150 V. S = 6. (46) Moist filter paper. "Responses" to 10 msec./150 V. and 100 msec./150 V. S = 47, 185.

responses to repetitive stimulation is limited by flash intensity because flash duration increases concomitantly and thus a flash tends to blanket or merge with preceding and succeeding peaks. The detection of frequency limits is also affected by the background glow that eventually develops. Assuming that the amount of glow is comparable in the two responses, and that flashes are approximately equal in intensity, the quick flash seems to have a markedly higher capacity for serial response than does the slow. The limit of 1:1 frequency response of the slow flash of the *Photuris* adult is 17–20/sec. (FF-I). The quick flash, in contrast, follows well at 40/sec. and apparently reaches its limit at something over 50/sec. Correspondingly, *Photinus pyralis,* which has real trouble in maintaining its slow flash at 4–5/sec., maintains its quick flash up to about 13/sec. For *P. punctulatus* the corresponding figures are 10 and more than 30/sec., and for *P. marginellus* they are 7 and 18/sec. These limits are roughly in the order of the respective species slow flash latencies (Table I). The contrast between the frequency compliances of slow flash and quick flash can be seen by comparing Figure 15 with Figure 13.

The quick flash is in general less stable than the slow and will not continue for hundreds or thousands of successive responses as may the adult slow flash. Nonetheless, under favorable conditions the fully facilitated quick flash may be quite reproducible (Fig. 16), making it possible to work out temperature effects and other data requiring repetitive stimulation. Fatigue or adaptation, when it does occur, may sometimes be overcome by intensifying the stimulus. For example, in tests on *P. marginellus* we found that flashing ceased after 20–30 successive responses to nominal 10 msec./150 V. stimuli given about a second apart, but would then resume at its original intensity for another 20–30 responses if the stimulus was changed to 20 msec./150 V., and so again at 30 msec./150 V.

Fatigue and adaptation also affect the interrelations of slow and quick flashes. Though the two flashes may occasionally be of equal magnitude (Figs. 1c, 7e) and may even facilitate together with repetitive stimulation (Fig. 17) it is more usual for one to come to predominate at the expense of the other (Figs. 7g, 7h). The quick flash seems to be favored by increasing either stimulus voltage (Fig. 5; Fig. 24e, f, g) or duration (Fig. 7). Changes may also occur with repetitive stimulation even without change in stimulus parameters. Usually it is the slow flash that drops out (Fig. 18), though if the stimulus is near the quick flash threshold the quick flash may disappear instead (Fig. 24a–d).

D. *Flash contour.* Quick and slow flashes may differ in form, but the fact that they may also resemble each other very closely, whether occurring separately (Fig. 25) or together (Fig. 7e), indicates that there is no *a priori* reason to suppose that the two effector events are basically different. A comparison of half-rise and half-fall times of quick and slow flashes of differing magnitudes likewise suggests that the two responses in a given individual are alike kinetically.

The quick flash, like the slow flash, may alter form somewhat under changing conditions of stimulation. There are some indications that the rise phase is slowed preferentially by low temperature and by increasing shock duration. The effects of increasing frequency have already been considered. Whether the continuous photogeny of the tetanized lantern or the background glow that is almost always present in quick flash experiments are comparable to any of the glow phenomena previously described (FF-I) is unknown.

3. *Flashing in relation to peripheral nerves*

Since nerves are inextricably and profusely incorporated in the lantern tissue there is no way, by stimulation alone, to test the possibility of peripheral nervous involvement in quick flash excitation. This problem was accordingly attacked by nerve section (see also Hanson, 1962).

In adult males of *Photuris* a transverse ventral cut was made at the boundary between abdominal segments 6 and 7 so as to leave segment 6 still innervated while destroying all connections between the nerve cord and the photogenic organ of segment 7. The animals were then carefully observed to make sure that spontaneous flashing still occurred in the segment 6 organ and not in the segment 7 organ. Then at intervals during the next 48 hours each segmental organ was tested separately for excitability, placing the electrodes directly in the photogenic tissue. In experiments with 15 specimens it was found that (a) the sixth segmental organ (control) of all individuals continued able to flash spontaneously and remained normally excitable for both slow and quick flashes throughout the experiment. (b) Up to about 20 hours after denervation, slow flashes with normal strength-duration relations, and also quick flashes, could be elicited from the segment 7 organ by appropriate stimuli (5 out of 5 specimens). (c) In the period 21–24 hours after operation, slow flash excitability was lost in 4 of 6 animals tested, quick flash excitability being retained in 5. (d) After 24 hours it was impossible, in the four remaining animals, to elicit a slow flash in the seventh segmental organ, though stronger stimulation did evoke the quick flash in each instance. As a control for the possibility that the loss of slow flash excitability in the denervated segment was due simply to inactivity of the lantern, a number of males were decapitated and tested for up to 8 days. They of course immediately ceased to flash spontaneously, but they remained able to produce both slow and quick flashes in both segmental light organs under appropriate electrical stimulation.

Since evidence from other insects indicates that it takes nerves a day or more to degenerate enough to lose their conductive powers, the present experiments could be interpreted to indicate that the normal slow flash is mediated by nerve, the quick flash not.

4. *Ultra-short latency responses*

A. *Larval flash.* In testing the isolated light organs of the *Photuris* larva to see if a short latency response similar to the adult quick flash could be induced, the effect of high voltage stimulation was found to be even more dramatic than in the adult (Fig. 26). The exact latency of the response is difficult to determine because of the low level of light emission but appears to be 1–3 msec. We shall refer to this response as the larval "flash" in distinction to the protracted normal larval "glow," which has a response latency of 600–800 msec. and a duration of 2–15 sec. (Fig. 27). Both flash and glow appear to involve the whole of the minute larval organ.

The larval flash facilitates with repetitive stimulation. The contrast in frequency compliance between short and long latency responses is even greater than in the adult, the normal glow being unable to follow stimuli one second apart (Chang, 1956; FF-I) whereas the flash shows clearly independent responses up to 10 stimuli per second (Fig. 28).

We have an occasional record indicating that the larval flash and larval glow may occur together—*i.e.,* records in which a temporary glow appears several hundred milliseconds after a flash. The strong stimulation commonly also induces a type of glow which begins at various brief intervals after the peak of the flash (Figs. 29, 30). Possibly this is analogous to the generalized glow that often follows intense stimulation of the adult lantern (FF-I, Figs. 63–66; FF-II, Fig. 43) and which may accompany intense serial stimulation of the larva (Fig. 31).

In some instances larval flash and glow seem to differ in contour, in that the flash shows a steeper rise than fall of luminescence (Fig. 26 vs. Fig. 27). In other instances (Fig. 30b) the flash resembles the normal glow in having an approximately symmetrical time course.

Larval flash latency seems to show the same relative independence of temperature as does that of the adult quick flash (Fig. 47).

In ten *Photuris* larvae the nerve leading to one of the pair of light organs was cut; then electrical excitability was tested in two larvae on each of days 1, 3, 5, 7 and 9 thereafter, with the normally innervated mate of each denervated

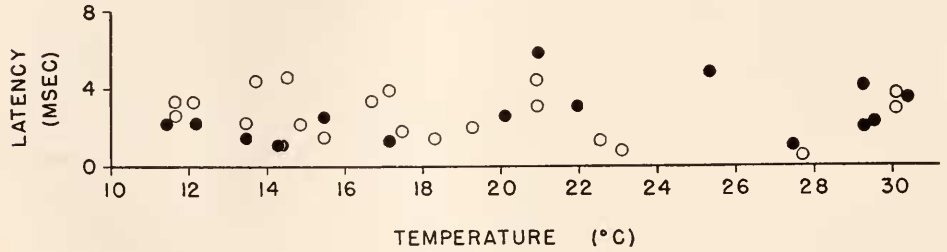


FIGURE 47. *Photuris* larval quick flash latency in relation to temperature. Thirty-six measurements on two individuals. Because of technical difficulties mentioned in text, the latency values are maxima.

organ serving as a control. Glows and flashes were both inducible on days 1 and 3, but after that only the flash was seen. It is concluded that the short latency response need not involve nerve.

B. *Adult quick-quick flash.* In experiments in which it was desired to exclude central nervous influence, lanterns were extirpated and laid across parallel electrodes in a moist chamber. With the small species, *Photinus consanguineus* and *P. marginellus,* the electrodes were often only about a millimeter apart because of small organ size. Under these conditions intense stimulation sometimes induced a dim and nearly instantaneous flash (Figs. 32, 34–38). This "quick-quick flash" was sometimes also obtained from large species of firefly if tissue fragments were used instead of whole lanterns (*P. punctulatus,* Fig. 40; *P. pyralis,* Fig. 41). The contrast between this response and the quick flash can be seen by comparing Figure 32 with Figure 33. Though sometimes occurring alone (Figs. 32, 38), the quick-quick flash usually merges with the quick flash either partially (Figs. 35, 36, 40) or completely (Figs. 34, 37, 41).

C. *Nature of ultra-short latency responses.* The fact that the flash of the larva and the quick-quick flash of the adult were only obtained with small pieces

of tissue and high stimulus intensities suggests two possible explanations: Either the responses are due to a genuinely different type of pathway of excitation from those previously described, or they represent an artifactual heating or ignition of the tissue.

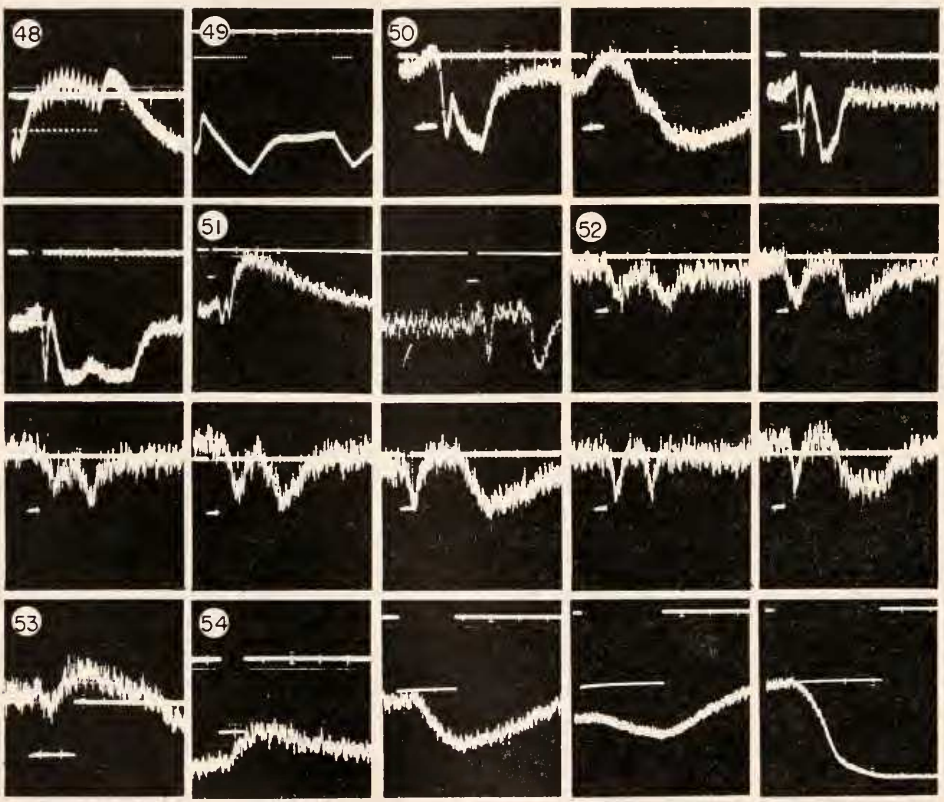
If the 1–3-msec. latency responses are biological rather than physical, the simplest explanation of the fact that they only appear under such special conditions would seem to be that the effect is one closely localized to the electrode, where the current flux is greatest. In other words, only when the total mass of responding tissue was small would the number of photocytes giving the very early response be large enough, in comparison with the number of cells giving slower types of response (quick or slow flash in adult; glow in larva), to become detectable. Detection would also be aided by the greater oscilloscope amplification necessitated by the small overall size of the tissue fragment. An analogous instance is the fact that a highly amplified record of a slow flash sometimes discloses a previously invisible leading shoulder (FF-I, Fig. 43). The fact that the ultra-short latency luminescences are always dim responses in the absolute sense is consistent with the idea that they reflect the activity of relatively few photocytes. Further, a biological nature for the quick-quick flash seems favored by its usual variability in relation to the quick flash (see above) and in relation to duration of stimulus pulse (Figs. 32, 34–40).

Considering now the artifact possibility, the fact that the quick-quick flash of some preparations can maintain its form practically unchanged through a dozen or more successive stimulations seems incompatible with the idea that a progressive charring of photogenic tissues is taking place. On a single cell basis the intensity of stimulation may be by no means as physiologically outrageous as might appear from the nominal stimulus parameters. However, in order to have an unequivocal artifact for comparison we "stimulated" small pieces of filter paper wet with saline. The response obtained, which we shall call a "spark," was of apparently zero latency but was quite different in some respects from most quick-quick flashes. For example, sparks were obtainable with stimulus pulses of 1 msec. or even shorter duration, which is never true for the quick-quick flash or larval flash. Also, the rise and decay phases of the luminescence were much faster than those of the responses obtained from photogenic tissue, so that duration was scarcely longer than the stimulus pulse (Fig. 45). With 10 msec. or longer stimuli sputtering occurred (Fig. 46) rather than the relatively long-lasting luminescence usual with tissue (Fig. 32, 34–41). However, in one lantern fragment preparation that had been used repeatedly, effects similar to sparks were obtained (Figs. 42–44).

If ignition of filter paper does occur, as suggested by Figures 45 and 46, the same effect probably would sometimes also occur in tissue. It seems possible that because of their extremely fast rise, 1–2-msec. duration and presumed limitation to a zone very close to the cathode, sparks would escape detection in records made at the oscilloscope sweep speeds ordinarily used and with appreciable masses of tissue. This might explain the absence of any spark-like element in Figures 32 and 34–41, while still allowing for occurrence of tissue sparks under extreme conditions (Figs. 42–44). However, it must be admitted that the biological significance of the quick-quick flash in the adult, and perhaps also of the larval flash, is questionable.

5. *Quenching phenomena*

In our first paper we showed that the continuous glowing that often develops in the lantern during and after vigorous repetitive induction of the slow flash can show either enhancement or depression both during and after electrical stimulation. The glows that arise during the evocation of quick and quick-quick flashes (see section 2C above) may likewise vary in intensity both spontaneously and as a consequence of stimulation, showing relatively slow enhancement and reduction (Figs. 48, 49) very reminiscent of the responses produced by weaker shocks in our previous investigation (*e.g.,* FF-I: Figs. 60, 65). We have also observed a curious phenomenon in the larva, in that baseline luminescence after the flash sometimes sinks to a lower level than preceding it (Figs. 26; 30c).



FIGURES 48–54. (48) *Photinus marginellus,* male, decapitated. Response to train of 10 msec./150 V. shocks at 10/sec. S = 3200. (49) As 48, but train frequency 15/sec. (two bursts). S = 4300. *Photinus marginellus,* female, decapitated. Four successive responses to 30 msec./150 V. S = 325. (51) *Photinus marginellus,* female, decapitated. Two responses to shocks of 10 msec./150 V. S = 425. (52) *Photinus marginellus,* male, decapitated. First, 5th, 6th, 9th, 13th, 14th and 15th successive responses to shocks of 10 msec./150 V. at 1.5 sec. intervals, after fatiguing. S = 175. (53) As 52 except 40 msec./150 V. S = 175. (54) *Photinus marginellus,* female, decapitated. Responses to 15 msec./150 V., 100/150 and 400/150. S = 130, 312, 625, 625.

From lanterns that have been so thoroughly fatigued by intense repetitive stimulation that they no longer flash, but show a steady dull glow, we have recorded some remarkable quenching responses that are comparable kinetically with flashes. Records from three individuals are shown in Figures 50–53. The response often begins with a slight increase in luminescence of near zero latency, presumably a quick-quick flash. After 5–20 msec. this is abruptly interrupted by the first quenching episode, which requires 10–15 msec. to reach maximum dimming (which is not complete extinction) and terminates in 20–25 msec. Almost immediately thereafter the light dims for a second time. Actually, it is unclear whether the overall phenomenon represents two brief quenchings or one prolonged quenching with a superimposed flash, but in any case the most interesting feature of the response is the initial short-latency dousing. The influence of stimulus duration on quenching is shown in Figure 54. Luminescence terminates in irreversible and complete "burnout" when the stimulus reaches 400 msec./150 volts (Fig. 54d). Like most of the other high voltage effects, the quenching changes are small in absolute intensity, suggesting a phenomenon closely localized to the electrode position.

Discussion

In our second paper we described a number of classes of long-latency response in the adult firefly, involving possible multiple cord conduction rates, central junctional delays, and central reverberation. In the present paper we have described two additional types of response, the quick and the quick-quick flashes, both with conspicuously shorter latencies than that of the normal slow flash. These responses need to be identified and integrated into a unified scheme of excitation. In attempting this synthesis from our exploration of a system hitherto essentially unknown electrophysiologically, we have had to adopt a somewhat more speculative approach than if the firefly lantern preparation had reached the conventional single unit nerve-junction-effector stage.

A. *Lantern microstructure.* Until quite recently it was not possible to advance much in the way of a concrete and coherent model of the excitation route in the firefly because the termination of peripheral nerve in the lantern was unknown and even the site of light production was in dispute. It is still not absolutely certain that the light is produced in, or at least only in, the so-called photocytes, although this is the traditional view and the one we shall adopt for present purposes. However, the innervation problem has been settled recently by the exquisite electron micrographs of Smith (1963) showing that the ultimate fiber, about 0.3 μ in diameter, does not touch photocytes at any point. Rather it terminates in a reticule-like ending containing both synaptic vesicles and neurosecretory-like droplets which embraces the mitochondria-filled tracheolar cell and is in turn embraced by the tracheal end-cell. This intricate structure, strategically situated just proximal to and in contact with the photocyte, near the transition point between terminal tracheal twig and ultimate tracheoles, has long been suspected of being involved in flash control though not as a neuroeffector link (see reviews in Buck, 1948; Hastings and Buck, 1956). For the purpose of our present speculations it will be assumed that this "end-organ" functions as a neuroeffector junction in the transfer of excitation in a three-element linkage: peripheral nerve-end organ-photocyte.

B. *Suggested nature of short-latency responses.* Considering, first, the identity of the adult quick flash, two main interpretations seem possible. Either it represents the response of a different group of photocytes from those producing the slow flash or it represents a response of the same cells triggered by a different process or pathway. We have not been able to exclude the former alternative but feel that essentially all our data favor the view that slow and quick flash represent the same effector event. These data include the close similarity of the two flashes in contour (Figs. 7e, 25), the apparent origin from the same tissue, and the mutual capacities for showing facilitation, summation, fatigue and other responses typical of a neuroeffector. The quantitative differences in threshold and in latency can be attributed to excitation differences.

The other short latency response of the adult firefly, the quick-quick flash, has much less secure physiological status than the quick flash, and we have far fewer data concerning it. Its usual kinetic difference from the induced filter paper spark, its variability, and its repetitivity, argue against its being a pure artifact. We suggest, primarily on the basis of its short latency and relative dimness, that it represents direct stimulation of a relatively small number of photocytes. We suggest the same explanation for the ultra-short latency larval flash. The fact that the larva lacks an intermediate latency response, corresponding to the adult quick flash, might seem an obstacle to the proposed scheme. Rather, it is a point in support, because the larval light organ differs from that of the adult in lacking tracheal end-organs.

We thus tentatively propose that slow, quick and quick-quick flashes all represent basically the same effector event; and that the overall response latency therefore comprises a delay representing peripheral nervous conduction, a delay involving nerve ending-end organ interaction, a delay due to activities of the tracheal end organ, and a delay during the ultimate excitation of the photocyte.

C. *Hypothetical origin of latency classes.* If, in the proposed chain of excitation, the thresholds to external stimulation of the three individual links are in the order photocyte > end organ > peripheral nerve, only nerve will be excited by a minimal stimulus. The latency of the eventual flash, involving conduction delay of nerve, junctional delays and photocyte activation time, will then be maximal (slow flash). If the stimulus strength is sufficiently increased, which can be accomplished either by increasing the voltage and/or stimulus duration or by reducing tissue mass, the thresholds of both end-organ and nerve to direct excitation will be exceeded. Thereupon two responses may occur. One involves the complete excitation chain (slow flash), and the other, skipping now the neural link, involves just the end organ-to-photocyte portion (quick flash). Finally, a still stronger external stimulus could by-pass both nerve and end organ, stimulating the photocyte directly (quick-quick flash). Depending on the interplay of stimulus strength, degrees of facilitation and fatigue, and tissue disposition in relation to electrode, the three responses might theoretically occur alone or in combination. Quick and slow flashes are usually easily differentiated instrumentally and in some instances (*e.g., P. pyralis*) even by eye. The quick-quick response, however, is not visually distinguishable from the quick flash, since the two latencies are so close, the intensity of the quick-quick flash so small, and the diffusion of light through adjacent non-excited photogenic tissue so prevalent. The quick-quick flash would, in fact, not be detectable even

in the oscilloscope record unless the light signal was strongly amplified or the tissue mass was sufficiently small that the directly excited photocytes in the region of most intense current flux close to the electrode formed an appreciable fraction of those excited through additional links.

D. *Evidence concerning by-pass hypothesis.* In evaluating the hypothesis that the three progressively shorter latencies are due to by-passing successive links in the excitation chain by increasingly intense stimulation, we shall use the approximate latency measurements from *Photuris* at 25°, namely 1 msec. for adult quick-quick flash and larval flash, 18 msec. for the quick flash, and 70 msec. for the slow flash.

The provisional identification of the quick-quick flash with direct excitation of the photocyte is supported only by the fact that its latency is of the right order for a process such as momentary depolarization of a membrane.

A distinction between quick and slow flashes is strongly supported by the nerve section experiments and the temperature data. The former indicate that the slow flash depends on nerve, the quick flash not. The latter shows that below 30° slow flash excitation changes exponentially with a Q_{10} of 2 or higher, whereas the quick flash Q_{10} averages around 1.4. If quick flash excitation were independent of conduction delay it would also be understandable why its frequency compliance can be so much higher than that of the slow flash. The 8-msec. quick flash latency is certainly inordinately long for junctional or end plate delays in known neuro-effector systems, although it might be reasonable for a neuro-glandular effect. Smith's study shows that the firefly end-organ is unique in that the nerve terminals are separated from the presumed ultimate effector, the photocyte, by another cell type, the peritracheal cell. However it seems unlikely that diffusion across even this gap of 1 μ or more (plus three cell membranes) could account for the otherwise excessive delay.

Turning finally, to the approximately 50-msec. differences between slow and quick flash latencies, this delay might represent either conduction alone or conduction plus some part of end-organ activation. The former is the simpler choice and it is by no means excluded, but we have already shown (Introduction) that there are considerable obstacles to assigning more than a few milliseconds of the total latency to pure conduction. In particular it would certainly be expected that some of the cells in a lantern responding to direct stimulation would be too close to the electrode to require anything like a minimum of 50 msec. for conduction of the stimulus to them. It seems likely, therefore, that the delay involved in overall end-organ and junction activation includes not only the 18 msec. suggested above but a considerable fraction of the slow flash latency. This implied complexity of excitation is consistent with the very complex end-organ morphology disclosed by Smith's study.

E. *Extinction mechanisms.* Historically, most of the attention to the firefly flash has been focused on the initiation and rise of luminescence, it having been assumed tacitly that the decay phase, like that of the *in vitro* "flash," merely reflects the chemical decay of an aliquot of reactants brought together by the excitation, or at most some sort of product or feedback inhibition. Buck (1948, 1955) did indeed speculate on the possibility that the firefly expends energy in keeping itself dark, flashing then representing a transient release from this inhibition, but this

idea was based on the uncontrolled glow that develops in hypoxia, anesthesia, and death, rather than on any specific neural mechanism. Although rise and fall of luminescence seem to be affected differently by temperature and hypoxia (FF-I, II), there are few indications that the falling phase of the flash is due to a specific extinguishing mechanism.

The rapid quenching phenomenon discovered in the present investigation, involving intense stimulation, fatigued tissue, and low-intensity light (hence probably an effect closely confined to the electrode region), is not likely to be related directly to the decay phase of the normal flash. As a reversible extinction mechanism it is nevertheless of great theoretical interest. Our previous demonstration of central inhibition of flashing by eye stimulation (FF-II) is irrelevant in the present connection, since it refers to a process that prevents the flash from starting, rather than one that snuffs out a glow already in being, but there would seem to be no *a priori* prohibition to some sort of peripheral inhibition, for example, one with a higher threshold and greater resistance to fatigue than the excitatory process.

The complex excitation path suggested by our findings indicates that study of single units will be necessary before much further progress can be expected. Preliminary studies have shown that the lantern tissue is held together so tenaciously by its profuse network of tracheoles that all usual dissociation methods fail. Hence it will probably be difficult to isolate single neuron-end organ-photocyte units. However, it has been found possible by localized stimulation to excite one or a few photocytes individually, and though these cells are inconveniently small (10 μ in largest dimension) it should be possible to record intracellularly.

Summary

1. Recordings of flashes from minute, widely separated regions of the lantern indicate that peripheral excitation is rather uniform.

2. Evidence is presented that peripheral conduction is unlikely to account for more than a small proportion of overall response latency.

3. By stimulating lantern tissue directly with high intensity shocks it is possible to evoke a much earlier response instead of or in addition to the normal flash. For example, in *Photuris* this "quick flash" occurs about 18 msec. after stimulation as compared with about 70 msec. for the normal or slow flash (at 25°).

4. The quick flash resembles the slow in contour, in apparently being produced by the same tissue, in showing a strength-duration effect for threshold and in showing facilitation, summation, adaptation, fatigue and other typical neuro-effector responses. The quick flash differs from the slow in its shorter latency, higher threshold, higher frequency compliance, and in having a relatively temperature-insensitive response latency.

5. Nerve section experiments show that the slow flash depends on nerves, the quick flash does not.

6. In the small larval light organ and in small pieces of adult lantern, very intense stimulation may induce a flash with a latency of the order of 1 msec. Control experiments with wet filter paper indicate that this "quick quick" flash is not entirely an artifact.

7. It is hypothetically proposed that stimulation involves a three-element neuro-effector (nerve terminal, end organ, photocyte) and that increasing strengths of

stimulus can by-pass in stepwise fashion certain links in this chain of peripheral excitation. According to this idea the ultrashort ("quick quick") response would represent direct excitation of the photocyte, the quick flash latency would represent delay necessary for excitation of the photocyte by the tracheal end organ, and the difference between slow flash and quick flash latencies would be time occupied in activation of the end organ complex plus a small delay for peripheral nervous conduction.

8. In lanterns subjected to long-continued intense stimulation the flash eventually disappears and is replaced by a steady glow. Under some circumstances intense shocks produce striking momentary double quenchings of this glow, kinetically similar to a flash. Peripheral inhibition is suggested as a possible mechanism.

LITERATURE CITED

Buck, John B., 1948. The anatomy and physiology of the light organ in fireflies. *Ann. N. Y. Acad. Sci.*, **49**: 397–482.

Buck, John, 1955. Some reflections on the control of bioluminescence. Pp. 323–332 in: The Luminescence of Biological Systems (Ed. F. H. Johnson), Amer. Assoc. Adv. Sci., Washington.

Buck, John, and James F. Case, 1961. Control of flashing in fireflies. I. The lantern as a neuroeffector organ. *Biol. Bull.*, **121**: 234–256.

Carlson, Albert D., 1961. Effects of neural activity on the firefly pseudoflash. *Biol. Bull.*, **121**: 256–276.

Case, James F., and John Buck, 1963. Control of flashing in fireflies. II. Central nervous aspects. *Biol. Bull.*, **125**: 234–250.

Chang, Joseph J., 1956. On the similarity of response of muscle tissue and of lampyrid light organs. *J. Cell. Comp. Physiol.*, **47**: 489–492.

Hanson, F. E., Jr., 1962. Observations on the gross innervation of the firefly light organ. *J. Ins. Physiol.*, **8**: 105–111.

Hastings, J. Woodland, and John Buck, 1956. The firefly pseudoflash in reaction to photogenic control. *Biol. Bull.*, **111**: 101–113.

Smith, David S., 1963. The organization and innervation of the luminescent organ in a firefly, *Photuris pennsylvanica* (Coleoptera). *J. Cell Biol.*, **16**: 323–359.